

What is Claimed is:

1. A method for increasing the expression levels of a *Neisseria* PorA protein or polypeptide in a host cell comprising the steps of:
 - 5 (a) infecting, transfecting or transforming a host cell with an expression vector comprising a polynucleotide comprising a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:24, wherein codon 18 is a codon other than an ATC;
 - (b) culturing the host cell under conditions suitable to produce the protein or polypeptide encoded by the polynucleotide of step (a); and
 - 10 (c) recovering the protein or polypeptide from the culture.
2. The method of claim 1, wherein the polynucleotide comprising the nucleotide sequence of SEQ ID NO:1 encodes a protein or polypeptide comprising an amino acid sequence of SEQ ID NO:2, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine residue.
- 15 3. The method of claim 2, wherein the polynucleotide encoding the PorA protein or polypeptide is isolated from *Neisseria meningitidis*.
- 20 4. The method of claim 1, wherein the polynucleotide comprising the nucleotide sequence of SEQ ID NO:3 encodes a protein or polypeptide comprising an amino acid sequence of SEQ ID NO:4, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine residue.
- 25 5. The method of claim 4, wherein the polynucleotide encoding the PorA protein or polypeptide is isolated from *Neisseria meningitidis*.
6. The method of claim 1, wherein the polynucleotide comprising the nucleotide sequence of SEQ ID NO:13 encodes a protein or polypeptide comprising an amino acid sequence of SEQ ID NO:14, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine residue.
- 30

7. The method of claim 6, wherein the polynucleotide encoding the PorA protein or polypeptide is isolated from *Neisseria meningitidis*.
8. The method of claim 1, wherein the polynucleotide comprising the nucleotide sequence of SEQ ID NO:15 encodes a protein or polypeptide comprising an amino acid sequence of SEQ ID NO:16, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine residue.
9. The method of claim 8, wherein the polynucleotide encoding the PorA protein or polypeptide is isolated from *Neisseria meningitidis*.
10. The method of claim 1, wherein the polynucleotide comprising the nucleotide sequence of SEQ ID NO:24 encodes a protein or polypeptide comprising an amino acid sequence of SEQ ID NO:25, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine residue.
11. The method of claims 10, wherein the polynucleotide encoding the PorA protein or polypeptide is isolated from *Neisseria meningitidis*.
12. The method of claim 1, wherein codon 18 is a TAC codon.
13. The method of claim 1, wherein the polynucleotide is operatively linked to one or more gene expression regulatory elements.
14. The method of claim 13, wherein one of the regulatory elements is a promoter.
15. The method of claim 1, wherein the vector is a plasmid.
16. The method of claim 15, wherein the plasmid is pET9a.
17. The method of claim 1, wherein the host cell is a bacterial cell.

18. The method of claim 17, wherein the host cell is *E. coli*.
19. The method of claim 18, wherein the *E. coli* is a DE3 lysogenic strain.
- 5 20. The method of claim 19, wherein the strain is selected from the group consisting of BLR(DE3)pLysS, BL21(DE3)pLysS, HMS174(DE3)pLysE and NovaBlue(DE3).
- 10 21. The method of claim 1, wherein the protein or polypeptide expressed is at least about 30% of the total cellular protein concentration.
22. The method of claim 1, wherein the protein or polypeptide expressed is at least about 50% of the total cellular protein concentration.
- 15 23. The method of claim 1, wherein the protein or polypeptide expressed is at least about 75% of the total cellular protein concentration.
24. An isolated PorA protein or polypeptide produced according to the method of claim 1.
- 20 25. An isolated *Neisseria meningitidis* polynucleotide comprising a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:24, wherein codon 18 is a codon other than an ATC codon.
- 25 26. The polynucleotide of claim 25, wherein codon 18 is a TAC codon.
27. An isolated *Neisseria meningitidis* PorA polypeptide or protein comprising an amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:14, SEQ ID NO:16 or SEQ ID NO:25, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine.
- 30 28. The polypeptide or protein of claim 27, wherein the amino acid at residue 18 is tyrosine.

29. A recombinant expression vector comprising a polynucleotide having a nucleotide sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:13, SEQ ID NO:15 or SEQ ID NO:24, wherein codon 18 is a codon other than an ATC codon.
5
30. The vector of claim 29, wherein codon 18 is a TAC codon.
31. The vector of claim 30, wherein the polynucleotide is selected from the group consisting of DNA, cDNA, genomic DNA, RNA and mRNA.
10
32. The vector of claim 31, wherein the vector is plasmid DNA.
33. The vector of claim 32, wherein the polynucleotide is operatively linked to one or more gene expression regulatory elements.
15
34. A genetically engineered host cell transfected, transformed or infected with the vector of claim 29.
- 20 35. The host cell of claim 34, wherein the cell is a bacterial cell.
36. The host cell of claim 35, wherein the bacterial cell is *E. coli*.
37. The host cell of claim 36, wherein the *E. coli* is a DE3 lysogenic strain.
25
38. The host cell of claim 37, wherein the strain selected from the group consisting of BLR(DE3)pLysS, BL21(DE3)pLysS, HMS174(DE3)pLysE and NovaBlue(DE3).
- 30 39. The host cell of claim 34, wherein the polynucleotide is expressed to produce the encoded polypeptide or protein.

40. An immunogenic composition comprising a *Neisseria meningitidis* PorA polypeptide or protein having an amino acid sequence of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:14, SEQ ID NO:16 or SEQ ID NO:25, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine.
5
41. The immunogenic composition of claim 40, wherein the amino acid at residue 18 is tyrosine.
- 10 42. The immunogenic composition of claim 41, further comprising one or more PorA polypeptides or proteins selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:18 and SEQ ID NO:20.
- 15 43. The immunogenic composition of claim 42, further comprising one or more adjuvants.
44. A method for identifying *Neisseria* polynucleotide sequences encoding PorA proteins or polypeptides which are expressed at low levels in a host cell, the method comprising:
20 (a) obtaining a mature *Neisseria* polynucleotide sequence; and
(b) determining the triplet sequence at codon 17, wherein an ATC at codon 17 indicates that the encoded PorA protein or polypeptide is expressed at low levels in a host cell.
25
45. An isolated polynucleotide identified according to the method of claim 44.
46. A recombinant expression vector comprising the polynucleotide of claim 45.
- 30 47. A genetically engineered host cell transfected, transformed or infected with the vector of claim 46.

48. A method for identifying *Neisseria* polynucleotide sequences encoding PorA proteins or polypeptides which are expressed at low levels in a host cell, the method comprising:

(a) obtaining an endogenous *Neisseria* polynucleotide sequence;

5 (b) determining the 5' signal sequence;

(c) hypothetically deleting the 5' signal sequence; and

(d) determining the triplet sequence at codon 17 of the sequence in step (c), wherein an ATC at codon 17 indicates that the encoded PorA protein or polypeptide is expressed at low levels in a host cell.

10

49. An isolated polynucleotide identified according to the method of claim 48.

50. A recombinant expression vector comprising the polynucleotide of claim 49.

15 51. A genetically engineered host cell transfected, transformed or infected with the vector of claim 50.

52. A method for increasing the expression levels of a *Neisseria* PorA polypeptide or protein in a host cell, the method comprising:

20 (a) obtaining a mature *Neisseria* polynucleotide sequence;

(b) determining the triplet sequence at codon 17, wherein an ATC at codon 17 indicates that the encoded PorA protein or polypeptide is expressed at low levels in a host cell; and

(c) replacing codon 17 with a codon other than an ATC.

25

53. The method of claim 52, further comprising step (d), adding a 5'-ATG codon to the sequence, wherein codon 17 in step (c) is now codon 18.

54. An isolated polynucleotide produced according to the method of claim 53.

30

55. The method of claim 53, further comprising the steps of:

(e) infecting, transfecting or transforming a host cell with an expression vector comprising the polynucleotide of step (d),

- (f) culturing the host cell under conditions suitable to produce the encoded protein or polypeptide, and
- (g) recovering the protein or polypeptide from the culture.

5 56. The method of claim 52, wherein replacing codon 17 in step (c) is a TAC codon.

57. An isolated polypeptide produced according to the method of claim 55.

10 58. An immunogenic composition comprising the polypeptide of claim 57.

59. A recombinant expression vector comprising the polynucleotide of claim 54.

15 60. A genetically engineered host cell transfected, transformed or infected with the vector of claim 59.

61. A method for increasing the expression levels of a *Neisseria PcrA* polypeptide or protein in a host cell, the method comprising:

- (a) obtaining an endogenous *Neisseria* polynucleotide sequence;
- (b) determining the 5' signal sequence;
- (c) deleting the 5' signal sequence;
- (d) determining the triplet sequence at codon 17, wherein an ATC at codon 17 indicates that the encoded protein or polypeptide is expressed at low levels in a host cell; and
- (e) replacing codon 17 with a codon other than an ATC.

20 62. The method of claim 61, further comprising step (f), adding a 5'-ATG codon to the sequence, wherein codon 17 in step (e) is now codon 18.

25 63. An isolated polynucleotide produced according to the method of claim 62.

64. The method of claim 62, further comprising the steps of:

- (g) infecting, transfecting or transforming a host cell with an expression vector comprising the polynucleotide of step (f),
- (h) culturing the host cell under conditions suitable to produce the encoded protein or polypeptide, and
- (i) recovering the protein or polypeptide from the culture.

5

65. The method of claim 61, wherein replacing codon 17 in step (c) is a TAC codon.

10

66. An isolated polypeptide produced according to the method of claim 64.

67. An immunogenic composition comprising the polypeptide of claim 66.

15 68. A recombinant expression vector comprising the polynucleotide of claim 63.

69. A genetically engineered host cell transfected, transformed or infected with the vector of claim 68.

20 70. A method for increasing the expression levels of a *Neisseria* PorA polypeptide or protein in a host cell, the method comprising:

- (a) obtaining a mature *Neisseria* polynucleotide sequence;
- (b) determining the triplet sequence at codon 17, wherein an ATC at codon 17 indicates that the encoded protein or polypeptide is expressed at low levels in a host cell; and
- (c) selecting an alternative *Neisseria* strain wherein codon 17 of the mature alternative strain sequence is a codon other than an ATC.

25

71. The method of claim 70, further comprising step (d), adding a 5'-ATG codon to the alternative *Neisseria* sequence, wherein codon 17 in step (c) is now codon 18.

30

72. An isolated polynucleotide produced according to the method of claim 71.

73. The method of claim 71, further comprising the steps of:

5 (e) infecting, transfecting or transforming a host cell with an expression vector comprising the polynucleotide of step (d),

(f) culturing the host cell under conditions suitable to produce the encoded protein or polypeptide, and

(g) recovering the protein or polypeptide from the culture.

10 74. The method of claim 70, wherein the alternative strain in step (c) has a TAC at codon 17.

15 75. An isolated polypeptide produced according to the method of claim 73.

76. An immunogenic composition comprising the polypeptide of claim 75.

20 77. A recombinant expression vector comprising the polynucleotide of claim 72.

78. A genetically engineered host cell transfected, transformed or infected with the vector of claim 77.

25 79. A method for increasing the expression levels of a *Neisseria* PorA polypeptide or protein in a host cell, the method comprising:

(a) obtaining an endogenous *Neisseria* polynucleotide sequence;

(b) determining the 5' signal sequence;

30 (c) hypothetically deleting the 5' signal sequence;

(d) determining the triplet sequence at codon 17 of the sequence in step (c), wherein an ATC at codon 17 indicates that the encoded protein or polypeptide is expressed at low levels in a host cell; and

(e) selecting an alternative *Neisseria* strain wherein codon 17 of the mature alternative strain sequence is a codon other than an ATC.

80. The method of claim 79, further comprising step (f), adding a 5'-ATG codon to the alternative *Neisseria* sequence, wherein codon 17 in step (e) is now codon 18.
- 5 81. An isolated polynucleotide produced according to the method of claim 80.
82. The method of claim 80, further comprising the steps of:
 - (g) infecting, transfecting or transforming a host cell with an expression vector comprising the polynucleotide of step (f),
 - 10 (h) culturing the host cell under conditions suitable to produce the encoded protein or polypeptide, and
 - (i) recovering the protein or polypeptide from the culture.
83. The method of claim 80, wherein the alternative strain in step (f) has a TAC at codon 17.
- 15 84. An isolated polypeptide produced according to the method of claim 82.
85. An immunogenic composition comprising the polypeptide of claim 84.
- 20 86. A recombinant expression vector comprising the polynucleotide of claim 81.
87. A genetically engineered host cell transfected, transformed or infected with the vector of claim 86.
- 25 88. A method of immunizing against *Neisseria* comprising administering to a host an immunizing amount of an immunogenic composition comprising a polypeptide having an amino acid sequence of SEQ ID NO:2, or a fragment thereof and a pharmaceutically acceptable carrier, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine.
- 30 89. The method of claim 88, wherein the amino acid at residue 18 is tyrosine.

90. A method of immunizing against *Neisseria* comprising administering to a host an immunizing amount of an immunogenic composition comprising a polypeptide having an amino acid sequence of SEQ ID NO:4, or a fragment thereof and a pharmaceutically acceptable carrier, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine.

5

91. The method of claim 90, wherein the amino acid at residue 18 is tyrosine.

92. A method of immunizing against *Neisseria* comprising administering to a host an immunizing amount of an immunogenic composition comprising a polypeptide having an amino acid sequence of SEQ ID NO:14, or a fragment thereof and a pharmaceutically acceptable carrier, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine.

10

15 93. The method of claim 92, wherein the amino acid at residue 18 is tyrosine.

94. A method of immunizing against *Neisseria* comprising administering to a host an immunizing amount of an immunogenic composition comprising a polypeptide having an amino acid sequence of SEQ ID NO:16, or a fragment thereof and a pharmaceutically acceptable carrier, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine.

20

95. The method of claim 94, wherein the amino acid at residue 18 is tyrosine.

25 96. A method of immunizing against *Neisseria* comprising administering to a host an immunizing amount of an immunogenic composition comprising a polypeptide having an amino acid sequence of SEQ ID NO:25, or a fragment thereof and a pharmaceutically acceptable carrier, wherein the amino acid at residue 18 is an amino acid other than an ATC encoded isoleucine.

30

97. The method of claim 72, wherein the amino acid at residue 18 is tyrosine.

99. A method of immunizing against *Neisseria* comprising administering to a host an immunizing amount of an immunogenic composition comprising a polypeptide having an amino acid sequence of SEQ ID NO:2 or a fragment thereof, a polypeptide having an amino acid sequence of SEQ ID NO:4 or a fragment thereof, a polypeptide having an amino acid sequence of SEQ ID NO:14 or a fragment thereof, a polypeptide having an amino acid sequence of SEQ ID NO:16 or a fragment thereof, a polypeptide having an amino acid sequence of SEQ ID NO:25 or a fragment thereof and a pharmaceutically acceptable carrier, wherein the amino acid at residue 18 of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:14, SEQ ID NO:16 and SEQ ID NO:25 is an amino acid other than an ATC encoded isoleucine.

100. The method of claim 99, wherein the amino acid at residue 18 is tyrosine.

101. The method according to any one of claims 44-53b, further comprising an adjuvant.

102. The method according to any one of claims 44-53b, further comprising one or more PorA polypeptides or proteins selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18 and SEQ ID NO:20.

103. An immunogenic composition according to any one of claims 42, 67, 76 or 85, further comprising one or more ORF2086 protein antigens comprising an amino acid sequence of SEQ ID NO:26 through SEQ ID NO:83.